AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Since 1825

Vol. 110. JULY, 1938 No. 7

CONTENTS

Annual Subscription, $3.00 Foreign Postage, 25 Cents Extra

Single Numbers, 30 Cents. Back Numbers, 50 Cents

Entered as Second-Class Matter at the Post Office at Philadelphia, Pa., Under the Act of March 3, 1879

Acceptance for Mailing at Special Rate of Postage Provided for in Section 1103 Act of October 3, 1917. Authorized February 15, 1920

EDITORIAL

On these pages the editor offers his opinions, unshackled by advertising patrons and unrestrained by anything save a sense of the decent and the truthful—the editor, alone, is responsible for their type, their tone and their tenor.

WHAT IS A POISON?

WHAT is a Poison?

Let us just seek the origin of the word and then attempt a definition.

It is generally accepted that the word came to us from the Latin, by way of the French. *Potio*—a Latin verb meaning "to drink," gives us the words pot, potable, potion and poison. On its road it suffered some mutilation for the Middle English had it as *puson, piuson* and *poyson.* The odd mutation of a perfectly harmless word such as potion into the much more significant word "poison" is not readily accounted for—except that the latter word came to be specially dedicated to a "deadly drink." Anyway we have in the modern English the two words—potable and poisonous, which despite a common origin are entirely dissimilar in meaning.

In an old French version of the New Testament, the good Samaritan is singularly described as a *poissoner,* thus giving to the uninitiated a perverted sense of the happy parable. Elsewhere we find that the old French and Middle English word *Pois*—meaning "to weigh out in small portions"—is claimed as the origin of the word poison.

Yet another alludes to the French word for fish, namely, *poisson,* as a possible source. Still it is natural to conclude that this word also came from the first-named word *potio* (to drink) for it is a well-known fact, anyway, that fish spend much of their time drinking.

While on the subject of etymology there is much that is interesting about the origin of some other words associated with poison. *Tox,* which is the syllable whence comes such words as toxicology (the study of poisons), intoxication, toxemia, antitoxin, etc., is of Greek origin. The Greek neuter noun *"toxon"* means a *"bow"* and in the plural was used for the bow and arrow and even for arrows alone. Arrows tipped with poisons, such as snake venom, have been used in

warfare since the dawn of history and the mutation of this Greek root as it came to us through the French, is thus readily accounted for. Indeed so recent an authority as the New English Dictionary defines the word Toxology—as the art of the bow and arrow.

It may be of further interest to note that the Hebrews use the same word to describe both the bow of warfare and the rainbow. To them that phenomenon was God's weapon forever hung high on clouds, a symbol and an earnest of His promise, never again to punish with flood and hurricane His sinning children.

"And it shall come to pass when I bring a cloud over the earth, that the bow shall be seen in the cloud and I will remember my covenant which is between me and you and every living creature of all flesh; and the waters shall no more become a flood to destroy all flesh."

And now comes an attempt at definition. Scientists have really never agreed on one definition. They rarely do on any definition. Every authority sports his own—and they are at great variance in some respects—and often incomplete. Here are a number of such definitions.

"Poisons are such inorganic or organic substances as are in part capable of artificial preparation, in part existing, ready formed, in the animal or vegetable kingdom, which, without being able to reproduce themselves, through the chemical nature of their molecules under certain conditions, change in the healthy organism the form and general relationship of the organic parts, and through annihilation of organs, or destruction of their functions, injure health, or, under certain conditions, destroy life" (Husemann).

According to Kobert—"Poisons are organic or inorganic unorganized substances originating in the organism itself, or introduced into the organism, either artificially prepared, or ready formed in nature, which through their chemical properties, under certain conditions, so influence the organs of living beings, that the health of these beings is seriously influenced temporarily or permanently."

Blyth prefers the following: "A substance may be called a poison if it is capable of being taken into any living organism, and causes, by its own inherent chemical nature, impairment or destruction of function." Sollmann states that, "A poison is any substance which, acting directly through its inherent chemic properties, and by its ordinary action, is capable of destroying life, or of seriously endanger-

ing health, when it is applied to the body externally, or in moderate doses (to 50 gms.) internally."

For obvious reasons the Law also *defines* a poison—and the Pennsylvania State Law embodies as its interpretation of a poison "a substance deleterious to life in doses of 60 grains or less."

Yet with all of these high-sounding explanations, for definitions they are most indefinite. Almost anything is a poison if taken in large enough quantities, and on the other hand there is scarcely anything that cannot be safely ingested if the quantity be minute enough. An old English definition, said to have originated with "Bluidy" MacKenzie, hated by good Scots everywhere, is as follows: "The best of droggis given in great excess is poyson, for poyson consists in excess of quantity as well as of quality, and whatever overpowers our nature." So ordinary a compound as common salt may promptly poison the human being if enough be indulged in at one dose. The Chinese frequently commit suicide by eating a cupful of salt, and even the Bible tersely bids us to remember Lot's wife, who died, it is said, of some such salification. One may eat four eggs for breakfast, and still live, but a teaspoonful of egg albumin, injected into the vein, will kill as certainly as strychnine. Even distilled water is fatal if much is so injected.

On the other hand, so active a substance as strychnine may not only be harmless, but actually a valuable medicine, if taken in sufficiently small doses. One grain of strychnine will kill the nine lives of one cat, but divided between ninety cats it becomes a mighty tonic —sleeks their fur, stimulates their purr and spreads their *meow* two octaves broader.

Then again, there is the element of time. Some poisons are cumulative, that is, they remain in the body, adding to their bulk, little by little, until the deposit finally becomes large enough to break the bank. Such a drug is arsenic. Certain vegetable drugs are said to act in a similar fashion.

And so we end our dissertation, still wondering what really constitutes a "Poison."

IVOR GRIFFITH.

ORIGINAL ARTICLE

THE BACTERICIDAL EFFECTIVENESS OF THE IMPROVED CALOMEL OINTMENT*

By F. W. Schiller

A NEW calomel ointment has recently been proposed by Vicher, Snyder, and Gathercoal (1), (2). The new ointment consists of the official base (4), into which a suspension of colloidal calomel has been incorporated. This ointment has been found to possess a bactericidal action far greater than that of the official calomel ointment. In fact, few ointments produce a broader zone in the F. D. A. Agar Plate Test than the new calomel ointment; of the official ones, only Citrine Ointment exhibits a greater activity in this respect.

The method for the preparation of colloidal calomel was developed by Broady and Jordan (3). The new calomel, consisting of particles 0.8 micron or less in size, was prepared by slowly adding, with constant agitation, a 3 per cent. solution of mercurous nitrate to an equal volume of a solution containing 2 per cent. of gelatin and 1.2 per cent. of sodium chloride. This suspension of calomel in gelatin was washed by dialysis and concentrated to contain 1 gm. of calomel in 3 gm. of the suspension. This yields a permanent suspension which can be incorporated directly into the ointment base.

The investigators found, by conductimetric determinations, the presence of ionic material in the filtrate of a suspension of official calomel in water. This may or may not be an explanation of the bactericidal action of calomel. Of great interest is the fact that a suspension of official calomel in gelatin solution has almost as high an antiseptic power as the new calomel suspension. Moreover, the investigators could offer no explanation as to how the reduced size of the colloidal calomel particles could so remarkably increase the bactericidal power of the ointment. The purpose of this paper is to in-

*Submitted to the Faculty of the Philadelphia College of Pharmacy and Science, in partial fulfillment of the requirements for the degree of Bachelor of Science in Chemistry.

vestigate the reason or reasons for its increased efficiency as evaluated by the F. D. A. Agar Plate Method.

Experimental

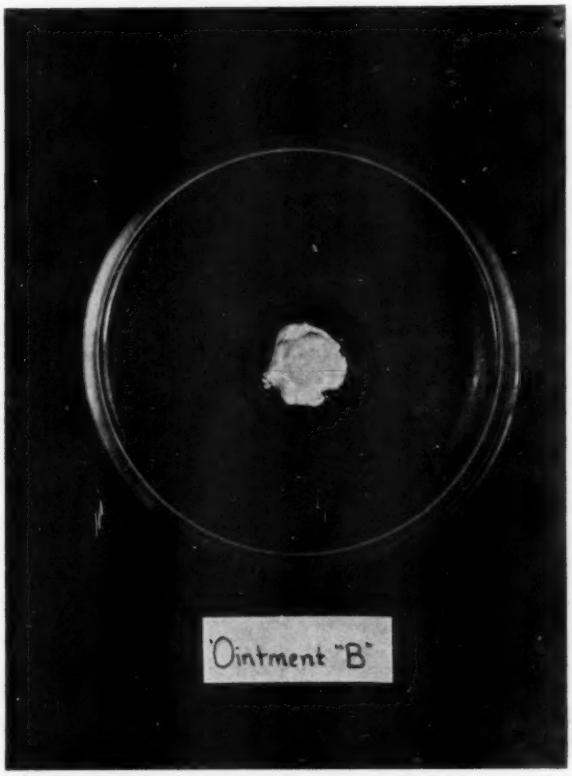
Upon investigation of this new colloidal calomel ointment it was found to differ from the official ointment in three ways: (1) the particles of calomel are colloidal, (2) the new ointment contains a greater amount of water, (3) the new ointment contains gelatin. It was thought that the increased activity was due not only to the colloidal size of the particles, but also to the increased water content. Further, it was thought that the increase in the amount of water present increased the ionization of the calomel, thereby yielding a greater number of mercurous ions available for distribution.

Accordingly, samples of the colloidal calomel ointment and a new ointment using a suspension of official calomel in water were made up. Also, an official calomel ointment was prepared. The methods were as follows:

Ointment B. Colloidal Calomel Ointment

The colloidal calomel suspension was first prepared by dissolving 9 gm. of anhydrous mercurous nitrate ($HgNO_3$) in 300 cc. of acidulated water (nitric acid 1 : water 100). Another solution was prepared consisting of 6 gm. of bone gelatin and 3.6 gm. of sodium chloride in 300 cc. of water. The mercurous nitrate solution was dropped from a separatory funnel into the sodium chloride-gelatin solution which was undergoing vigorous mechanical agitation. The precipitate of colloidal calomel was dialyzed in a parchment bag for 24 hours. The dialyzate was tested at this time and found to be free of acid and soluble salts. The suspension was then concentrated by vacuum evaporation. The calomel suspension after concentration was found to contain 54.3 per cent. calomel by weight. This then was diluted to yield a 33.3 per cent. suspension of colloidal calomel.

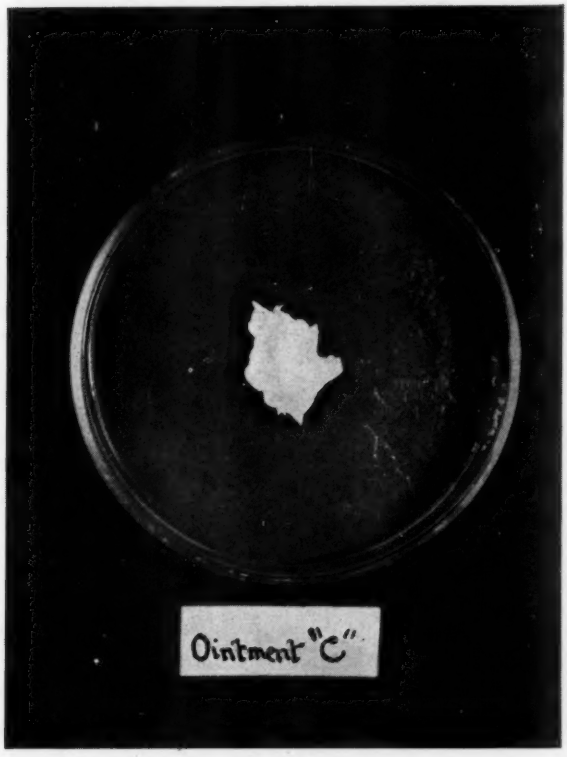
The ointment was prepared using 18 gm. of suspension and incorporating this into a base consisting of 7 gm. of white petrolatum and 7 gm. of hydrous wool fat. Difficulty was encountered in emulsification as the base would not take up the water. Accordingly, 1 gm. of an oxy-cholesterol base was added and the amount of the calomel suspension increased in order to keep the proportion constant. The ointment so made contained 18 per cent. of colloidal calomel by weight.



Ointment C.

This ointment was prepared as follows:

The suspension was made by triturating 6 gm. of official calomel with 12 gm. of water. This was then allowed to come to equilibrium for 24 hours. The 18 gm. of suspension was incorporated into 14 gm. of a base consisting of equal parts hydrous wool fat and white petrolatum. In this instance it was not necessary to add an oxycholesterol base, the official base readily taking up the water. This observation was later found to be of considerable significance. The ointment so prepared contained 18 per cent. of official calomel by weight.

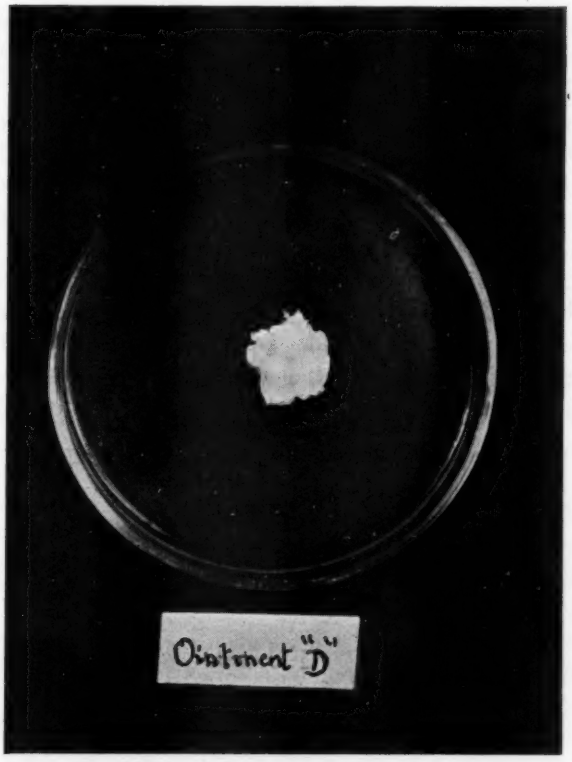


Ointment D. Calomel Ointment N. F. VI.

An official ointment was prepared by incorporating 6 gm. of official calomel into 14 gm. of a base consisting of equal parts hydrous wool fat and white petrolatum.

Antiseptic Properties of the Calomel Ointments

Tests for the bactericidal effectiveness of the ointments were run according to the F. D. A. Agar Plate Technic (5). In this work 100 mm. petri dishes provided with earthenware covers were used. After the melted agar had been inoculated with a culture of staphylococcus aureus and the plates poured and hardened, the samples of ointments were placed in intimate contact with a small surface area of the inocu-



lated agar. After incubation for 24 hours, the width of the inhibition zone was measured (Plates I, II, III).

The following tabulation gives the results of these tests:

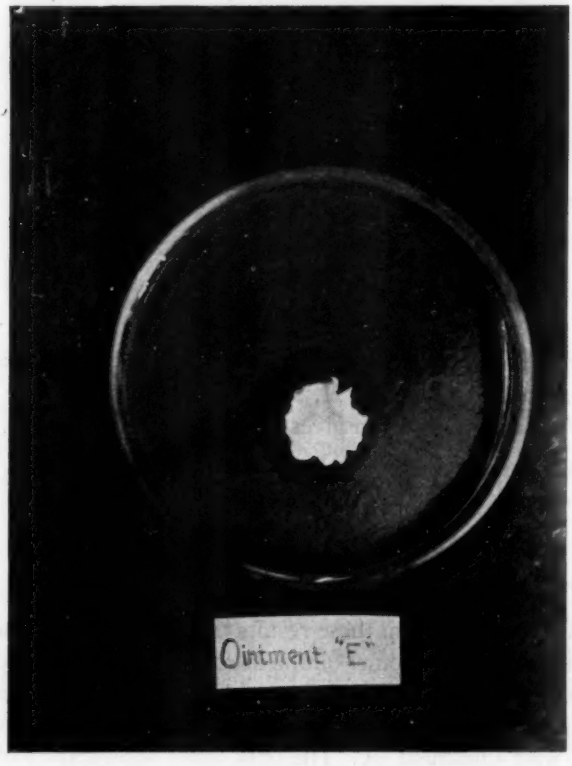
Ointment	Width of Inhibition Zone
B	9.0 to 11.0 mm.
C	1.0 to 2.5 mm.
D	0.5 to 2.0 mm.

It was concluded from these results that the increased bactericidal action of the colloidal calomel ointment could not be explained on the basis of its increased water content, although there was some slight increase in the width of the zone of inhibition with Ointment C as compared with Ointment D. The only other significant variable not

yet studied was the presence of gelatin in the colloidal calomel ointment. Accordingly a fourth ointment was prepared as follows:

Ointment E.

A 6 gm. sample of official calomel was triturated with 12 gm. of a 1 per cent. bone-gelatin solution. This suspension was allowed to come to equilibrium for 24 hours and was then incorporated into 14 gm. of a base consisting of equal parts hydrous wool fat and white petrolatum. Difficulty was again encountered in the emulsification and it was concluded that this must be due to the presence of gelatin, since such difficulty was not experienced in preparing Ointment C. It was therefore necessary to add 1 gm. of an oxy-cholesterol base and then enough of the calomel suspension to keep the proportion constant, namely 18 per cent. of official calomel by weight. This new Ointment



E was now tested as heretofore described together with B, C, and D. The N. F. VI base and also the oxy-cholesterol base were plated to determine their bactericidal efficiency, if any. The results of these were as follows:

Ointment	Width of Inhibition Zone
B	9.0 to 11.0 mm.
C	1.5 to 2.5 mm.
D	0.5 to 1.5 mm.
E	7.0 to 11.0 mm.
N. F. VI base	none
Oxy-cholesterol base	none

From a survey of these results it would appear that the presence of gelatin was the deciding factor in producing a wide zone of inhibition. The new ointment (Plate IV) was approximately equal in bactericidal efficiency to the colloidal calomel ointment.

The possible bactericidal action of the gelatin was checked by adding 0.1 gm. of gelatin to 9 cc. of bouillon containing 0.1 cc. of a 48 hour old culture of staphylococcus aureus. This was incubated for 24 hours and the gelatin was found to have no bactericidal action. It was found by experiment that further increase in the gelatin content of calomel ointments did not increase their bactericidal power.

Conclusions

According to these findings it is believed that the increased effectiveness of the new colloidal calomel ointment is largely dependent upon the greater availability of water (containing mercurous ions) in the ointment.

This greater availability is probably due to emulsion antagonism; gelatin favoring an oil-in-water dispersion as against lanolin which favors a water-in-oil. Thus the presence of gelatin results in the water being less firmly held by the lanolin base.

It seems quite unlikely that the zone of inhibition on the agar plate could be produced other than by the penetration into the agar gel of water containing dissolved calomel and mercurous ions. It is not to be expected that either the base or solid calomel particles could penetrate far into the body of such a hydrophilic gel during the relatively short interval of testing.

The increased efficiency of calomel ointment either colloidal or in the presence of gelatin may be only an apparent one since it is based entirely on the agar plate test. It is quite possible that the ointment in actual prophylaxis may possess no greater efficiency than that heretofore used.

On the basis of this study it seems unnecessary to follow the rather lengthy process of preparing a colloidal calomel suspension, inasmuch as an ointment may be prepared using a suspension of calomel in a 1 per cent. gelatin solution which appears to be equally effective when tested by the agar plate technic. It would seem that some further tests are required to prove the superiority of the colloidal calomel over the method described above.

Summary

I. An investigation as to the cause of the increased efficiency of the Improved Calomel Ointment is reported.

II. On the basis of experimental results it seems evident that the presence of gelatin in the suspension is a factor of foremost significance in producing a wide zone of inhibition when ointments containing such suspensions are tested by the F. D. A. agar plate technic.

III. A considerably simplified method of preparation is suggested which, unless further evidence to the contrary is obtained, would seem to provide an equally effective product.

Acknowledgment

In presenting this thesis, the author wishes to sincerely thank Professor Linwood Tice for his invaluable assistance and guidance.

REFERENCES

(1) Vicher, E. E., Snyder, R. K., and Gathercoal, E. N.: "An Improved Calomel Ointment," *N. F. Bull.*, VI, 2699 (1937).

(2) Vicher, E. E., Snyder, R. K., and Gathercoal, E. N.: "An Improved Calomel Ointment," *Jour. A. Ph. A., 26,* 1241 (1937).

(3) Broady, P., and Jordan, C. B.: "Some Applications of Colloidal Chemistry to Pharmacy," *Ibid., 16,* 425 (1927).

(4) *National Formulary,* VI.

(5) Ruehle, G. L. A., and Brewer, G. M.: "United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants," *U. S. Dept. of Agriculture Circular,* No. 198 (1931).

THE RESPONSES OF DAPHNIA MAGNA TO VITAMIN E

By Arno Viehoever and Isadore Cohen [1, 2, 3]

Philadelphia College of Pharmacy and Science

CONTENTS

[1] Supported by a research grant from Merck & Company.

[2] A contribution from the Gross Laboratory for Biological and Biochemical Research.

[3] Presented before the Medical Section of the American Association for the Advancement of Science in Ottawa, Canada, on June 27, 1938.

I. INTRODUCTION

THE value of *Daphnia magna,* the transparent crustacean, as an ideal test animal in experimental biological work has been established previously in studies of drug evaluation and standardization. The plasticity of response of Daphnia to cultural conditions combined with the ease of observing the growth of ovaries and of the parthenogenic embryos (virgin birth) in the brood sac encouraged us to study the responses of *Daphnia magna* to the presence and absence of vitamin E in their propagation.

The role of vitamin E in invertebrate physiology, has to our knowledge, not been adequately studied. It was felt that our findings might conceivably have a definite bearing upon the general problem of physiological similarity and dissimilarity between vertebrates and invertebrates. In previous studies we have found that the responses of *Daphnia magna* to drug action resembled very closely those shown by vertebrates. Moreover, it was suggested that a more rapid method for the quantitative determination of potency of vitamin E, now prepared synthetically, would be desirable for replacing the slow method in current use.

II. CULTURAL METHODS

The propagation of *Daphnia magna* has been reported in 1935 by Viehoever (9). For several years large numbers of daphnia have been grown for experimental studies dealing with the mechanism of drug action: strychnine (10), laxative substances (11), elaterin and cascara which are now in progress, aphrodisiac and irritant substances (13), and, finally, for current work in the testing of digitalis preparations, marihuana (12), toxins and venoms.

Bovung (dried, shredded cow manure), Wizard sheep manure (dried, shredded) and Cellu soy bean flour with urea added have been quite satisfactorily used until recently as nutrient sources. However, critical cultural experiments have revealed important differences between them.

The manure media are made up per gallon by first boiling 2 grams of Bovung or Wizard in 50 cc. water which is then added to the water containing a piece of marble. After fermentation and inoculation of fodder organisms from a successful culture, the media are ready for use after three days. Boiling the manure prevents the development of parasitic flat worms whose eggs might be present.

The standard soy bean medium used is made up by adding 500 mg. Cellu and 100 gm. urea to a gallon of well-aerated, dechlorinated tap water containing a piece of marble. This can be used on the day following inoculation.

The daphnia used in the study of the mechanism of drug action were standardized in respect to age, sex, cultural and environmental history in the following manner. Gravid females were isolated in one ounce bottles, containing the culture medium, and checked daily. Those which had released young were transferred to a new bottle while the young were all placed together in a gallon jar, half filled with culture media and containing a piece of marble. The cultures were placed in northern light and the temperature was maintained at 70 degrees F.

In contrast to the nutritional studies made upon economically important animals (stock, poultry, fish) and man, very little work has been conducted upon invertebrates. An analysis of "Culture Methods for Invertebrate Animals" (3), reveals that practically all such cultural methods are empirical and merely attempt to simulate natural conditions. Hence attempts to grow daphnia in vitamin E-free culture media necessitated exploratory experiments for the obtaining of such nutrient. Extraction with petroleum ether of the fat soluble constituents of the manures and soy bean flour was attempted. A supposedly vitamin E-free food mix,* designed for rat studies, was obtained from the Harris Laboratories.

*The formula is as follows:

Casein-Harris free from all vitamins	18%
Salt mix	4%
Fat-free brewers' yeast (ether extracted)	10%
Cod liver oil (assayed)	1%
Olive oil	9% (?)
Corn starch (vitamin free)	58%

III. REPRODUCTIVE RHYTHM OF DAPHNIA MAGNA

The ovaries of *Daphnia magna* can be seen on the fifth day under optimum cultural conditions at 70 degrees F. The oöcytes and their germinical vesicles are clearly visible. The ovaries are paired structures lying on each side of the intestine. The oögenic tissue of the ovaries is located within the region where the intestine makes a descending curve before entering into the rectum.

Four oöcytes are developed but only one is destined to grow further at the expense of the other three. These oöcytes have a diploid chromosome number. Since reduction and fertilization had not taken place the young are products of diploid parthenogenesis. These eggs are commonly known as summer eggs in contrast to winter eggs (epiphibia) in whose production the males participate. Having bred this strain of *Daphnia magna* in the laboratory for over ten years, there is no question as to their genetical uniformity.

With the further proliferation of oöcytes and with the growth of those already cut off, the ovaries extend alongside of the intestine as far anterior as the heart. At the end of the sixth day there is increase in size and appearance of nutritive material which assumes a bluish green coloration. This is an advanced stage in ovarian development and between the seventh and eighth day the embryos, having the same coloration, are released into the brood sac. Here they undergo further their embryological development rapidly, and on the tenth day are released into the environment, self-sufficient. (See Plate I.)

Plate I

Reproductive Rhythm of Daphnia Magna

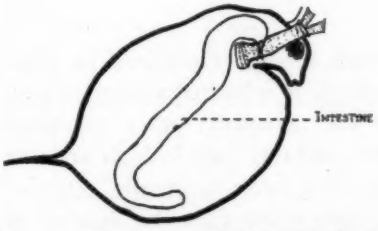


Fig. 1. Negative Ovarian Growth (—)

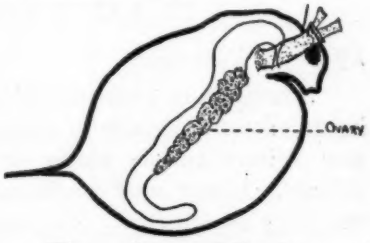


Fig. 3. Advanced Ovarian Growth (++)

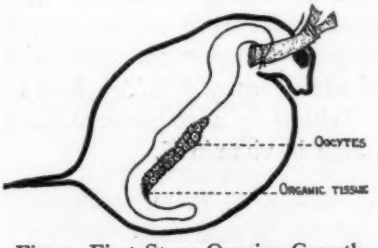


Fig. 2. First Stage Ovarian Growth. Oöcytes Visible (+)

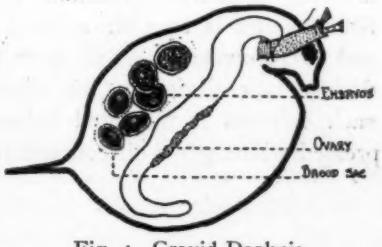


Fig. 4. Gravid Daphnia

Increase in temperature may speed this entire sequence through in nine days but the animals are short lived. A decrease in temperature and optimum nutritive conditions will prolong the time of the ovarian development and the sequence as shown by our experiments.

Under optimum cultural conditions, as soon as the embryos are released into the brood sac, the ovaries repeat their cycle. Upon the release of the young from the brood sac, the matured ovaries are again ready to release additional embryos into the brood sac. In many instances it has been observed that such release may occur within three hours after the discharge of the fully developed young from the brood sac. As a rule, the release of subsequent clutches into the brood sac does not occur for several hours. Every third or fourth day, a new brood of young may be expected.

In the subsequent releases there is a sharp increase in the number of young. This is correlated directly with the general increment of growth in the mother. As high as fifty young per brood have been counted. However, such fecundity and frequency of rhythm declines with advanced age, the lifespan or expectancy of *Daphnia magna* being approximately ninety days.

IV. EXPERIMENTAL RESULTS

Effect of Starvation

Starvation of experimental animals is readily obtained by placing an excessive number of young animals in a limited volume of regular Wizard culture media or in the petroleum ether extracted Wizard. Under such conditions the mortality rate is high and the survivors are stunted. The reproductive cycle is greatly retarded or absent. If ovarian development does occur then the number of young released is small, seldom more than two per clutch. Under favorable nutritive conditions, conspicuous storage of colored, fat-like globules is seen throughout the daphnia, while in the starved and overcrowded ones, no such storage is apparent and there is a decided lack of vigor. The effect of adding vitamin E rich food to such cultures is tabulated below. Triticol is a commercial, cold pressed wheat germ oil concentrate, high in vitamin E.

TABLE I

OVARIAN DEVELOPMENT OF STARVED DAPHNIA IN PRESENCE OF FOOD RICH IN VITAMIN E (TRITICOL)

Age of Culture	Condition of Animals	25 mg. of 5 gm. Cellu + 10 drops of Triticol		
		2 days	3 days	4 days
20 days	8— stunted	——	4— 3++ 1 dead	2+)* 4++) 1 gravid
13 days	9— stunted 3 gravid, medium size	8+ 1—**		
12 days	3— medium size	1+ 2++	3++	
11 days	10—*** 2+	1— 6+ 3 dead	5+ 2++	
10 days	13—*** 7+ 1++	12+ 1++		
9 days	23—	10— 13+	23+	

No visible ovarian development = —; oöcytes visible = +. Green color in ovaries = ++.

*Animals doubled in size.

**Appears sickly, low heart beat.

***Only animals with ovarian development absent were used.

Effect of Stagnation

It has been our experience that in old cultures of the Bovung type the reproductive rate of the daphnia is low, if present at all. This is an indication of stagnation, where, possibly, the exhaustion of optimum quantities of nutritive material has taken place. The addition of vitamin E rich food rapidly restored the fertility of such animals. When animals from a stagnant culture were closely studied,

it was discovered that in some instances their ovaries would develop to the stage when the individual oöcytes were visible and then regress or become reabsorbed. The beneficial action of vitamin E rich food upon stagnated daphnia is illustrated by the results of this representative experiment.

Twenty-four daphnia of equal size and with no visible ovarian development were selected from a stagnant Bovung culture. Twelve daphnia were placed in a museum jar containing 8 ounces of the same medium, while 25 mg. of the Cellu-Triticol mixture were added to the other twelve animals in a similar volume of medium.

TABLE II

OVARIAN DEVELOPMENT OF DAPHNIA FROM STAGNANT BOVUNG CULTURE TREATED WITH VITAMIN E RICH FOOD

	2 days	3 days	4 days	5 days	6 days
12 Control daphnia with no ovarian development	6— 2+ 1 gravid* 3 dead	6— 2+ 1 gravid	3— 3+** 2 gravid	6—** 1 gravid 1 dead	6— 1 gravid
12 Experimental daphnia in same condition	5— 7+	2— 7+ 2 gravid 1 dead	1— 4+ 5 gravid 1 dead	2+ 8 gravid	10 gravid*** 3 young

*Embryo small. Ovarian condition mistaken during initial inspection.
**Ovarian regression in at least two daphnia.
***Average number of embryos per animal = 5.6.

Deficient Culture Media

A supposedly vitamin E-free food purchased from the Harris Laboratories, high in oil content, yielded variable results when attempts were made to use it as the basic component of a culture medium. After several trials, four-day old daphnia, grown on the Cellu-urea medium were established. These remained small and only one of several dozen released an embryo into the brood sac. The other animals appeared starved. After twenty days they all died. The media contained numerous fat globules. In another trial, where

the culture medium had been permitted to ripen for twelve days, an inoculation of young animals from a Cellu-urea culture resulted in a culture with a ten-day reproductive cycle. The animals released several embryos into the brood sac and appeared to be vigorous. However, the culture soon became stagnated and all the animals suddenly died.

Another instance of starvation and stunting of daphnia cultures was encountered in the use of Michell sheep manure. The mortality rate was high and most of the animals in a culture remained stunted. Daphnia that finally attained sexual maturity released but few young in the initial and following clutches.

Effect of Extracted Culture Material

As part of our experiments in attempting to achieve a vitamin E-free culture medium, Bovung cow manure, Wizard manure and Cellu soy bean flour were extracted with petroleum ether. Cultural experiments show that Bovung thus extracted supplies a more adequate nutritional source than Wizard, while, in the case of Cellu, the cultural results obtained were of a variable character.

Bovung versus Wizard

Under similar conditions, daphnia, born and raised in extracted Bovung, released embryos into their brood sacs after eleven days, and were twice the size of the animals grown in extracted Wizard. Furthermore, the latter animals do not develop ovaries or release young for well over a month after birth. Further studies between the two manures clearly show the nutritional superiority of Bovung over Wizard.

The brood mothers, which had been used as a source of supply for young born in the extracted manure media, were placed together respectively into mass cultures. These brood mothers, selected from a regular Bovung culture, were originally of the same size and with approximately equal numbers of embryos released in the brood sac. After one week, seven gravid animals were taken at random from the extracted Bovung mass culture and the number of embryos were counted. Their numbers were 12, 12, 12, 10, 10, 8, for an average of 10.2. Similarly, seven animals were taken from the extracted Wizard mass culture but isolated only with difficulty since most of the daphnia, comparable in size to those from the Bovung culture,

were non-gravid. Their numbers were 6, 6, 6, 4, 2, 2, 2, for an average of 4.

An alternative possibility might be advanced that Wizard sheep manure may not be deficient in proper nutriment but contains less than Bovung cow manure per unit weight. From the results of the following comparison, this possibility indicated the desirability for further investigation.

Superiority of Bovung over Wizard as a Nutritional Source

Twenty gravid 15-day old daphnia were selected from a regular Bovung culture for conformity in size and number of embryos in the brood sac. Ten were placed in a newly prepared Bovung culture medium and ten in. Wizard, both media made up with two grams of manure per gallon. The Bovung culture medium was slightly cloudy at the start while the Wizard was clear, this indicating a lessor degree of pollution in the latter. The degree of initial pollution might well indicate the availability of certain substrates upon which the fodder organisms thrive. After a few days the Bovung culture medium cleared but many animals from the first clutch of releases died.

After two weeks, it was apparent from merely gross inspection that at comparable concentrations Bovung does supply a more favorable medium than Wizard sheep manure for the propagation of daphnia. The surviving original daphnia in the former were of giant size with 30-50 embryos in the brood sac, while those in Wizard were decidedly smaller with only 2-8 embryos. Differences in coloration, which is apparently related to nutritional factors, were clearly discernible between the animals of the two cultures. A microscopic examination of the animals showed that there was a profuse storage of fat-like colored globules throughout the bodies of Bovung cultured daphnia, while such storage was lacking in those cultured in the Wizard manure.

After eighteen days the experiment was terminated and the following count made.

Bovung

6 extra large, originals gravid with 30-50 embryos
45 large, majority gravid with many embryos
50 medium
350 small, conservatively estimated.

Wizard

4 large originals, gravid with but few embryos, intermediate between extra large and large of Bovung
47 medium, non-gravid
88 small

However, the possibility that Wizard contains less nutritive matter per unit volume than Bovung can be excluded by the results of the following experiment.

Ten medium size daphnia were taken from the above described Wizard culture and placed in culture medium made up with 2, 4 and 6 grams of Wizard per gallon. Thirty days later the following count was obtained.

Effect of Concentration on Growth

	Large	Medium	Small
2 gm. Wizard	9	75	150 (est.)
4 gm. Wizard	2	40	250 (est.)
6 gm. Wizard	0	8 (original)	20

It can readily be seen that while 4 gm. concentration of Wizard appears slightly better than the 2 gm. of Wizard, it does not at all compare favorably with the 2 gm. concentration of Bovung. At 6 gm., the salt concentration inhibits actual growth and propagation of the culture. On the basis of these experiments it is demonstrated that Daphnia respond to nutritive cultural differences.

Cellu Media

The cultural results of experiments made with petroleum ether extracted Cellu soy bean flour, 0.5 gm. per gallon, are not as clear cut as in the case of Bovung and Wizard manures. In one instance, three-day old animals from a regular Cellu culture did not thrive in this medium and died after eleven days, while in other instances good growth was obtained. However, these cultures rapidly stagnated. Incomplete extraction and the presence and absence of different types of fodder organisms may well explain the variability of results.

Effect of Vitamin E Rich Culture Medium

The presence of vitamin E in appreciable amount in culture media is readily detected by the rapid restoration of ovarian dys-

function combined with the increased growth and vitality of the test animals. When daphnia are cultured on petroleum ether extracted Wizard sheep manure, their reproductive cycle is retarded, their fertility is of a low order and their growth impaired. If placed in media containing Triticol such animals respond definitely in two days, provided that they are not too severely stunted.

Restoration of Ovarian Function

Forty-four animals with no visible ovarian development were selected from a mass culture grown on petroleum ether extracted Wizard sheep manure, 2 grams per gallon. These were placed into culture medium made up with the same concentration of extracted Wizard but containing 4 drops of Triticol which was first absorbed upon the dry manure. The results were as follows:

Start: 44--*	
2 days	8+, 30++, 5 gravid, 1 dead
3 days	3+, 21++, 14 gravid, 6 dead

Similar results were obtained with animals whose previous cultured history was known.

From an extracted Wizard culture containing thirteen animals which were sixteen days old, four animals were placed in two museum jars containing the extracted Wizard culture medium with added Triticol, while the other five daphnia were placed in a museum jar containing the original medium. These animals had not previously shown any ovarian development. The results obtained over a period of eight days clearly show the effects of vitamin E rich culture medium upon sexually retarded daphnia.

Ten severely stunted 15-day-old animals grown in the extracted Wizard were placed at the same time in this vitamin E rich medium and similarly observed. For three days there was no apparent growth. However, when examined microscopically, a profuse storage of fat-like globules was seen to be taking place. Upon the fourth day marked growth was clearly discernible although the ovarian development was still negative. On the fifth day there was a positive ovarian development in all ten animals and the further development parallelled that of the above recorded animals.

*No visible ovarian development = —; oöcytes visible = +; green color in ovaries = ++.

TABLE III

EFFECT OF VITAMIN E RICH FOOD ON 16-DAY OLD SEXUALLY RETARDED DAPHNIA, PETROLEUM ETHER EXTRACTED WIZARD

	With Triticol		Without Triticol
Days	Exptl. Jar I 4 daphnia	Exptl. Jar II 4 daphnia	Control 5 daphnia
Start	4—*	4—	5—
2 days	1+, 3++	2+, 2++	4—, 1+
3 days	3++, 1 gravid	4++	3—, 1+, 1++
4 days	3++, 1 gravid	3++, 1 gravid	3—, 1+, 1++
5 days	4 gravid (6 1, 5, 5)	1++, 3 gravid (6, 5, 8)	2—, 1++, 1 gravid (1)
6 days	1++, 3 gravid, 1 young	4 gravid	1—, 2+, 1 gravid
7 days	4 gravid	4 gravid, 3 young	1—, 2+**, 1 gravid
8 days	4 gravid, 8 young	4 gravid, 7 young	4—**, 1 young

Further Extraction Experiments

In an attempt to extract completely all fat soluble constituents from Wizard sheep manure, which comes shredded, the manure was finely ground, pulverized and sifted. Only the fine powder was extracted twenty times with petroleum ether, while the coarser elements were discarded. Instead of further decreasing the fertility of daphnia grown in it in mass culture, there was a puzzling increase of fertility. The color of this culture medium took on a deeper shade on standing than the extracted, shredded Wizard. It is conceivable that the greater surface obtained per unit weight after grinding may well afford an actual increase of available substrate for a more abundant development of fodder organisms, upon which the daphnia feed. However, in restricted cultures this fertility soon disappears thus indicating its threshold character.

A comparison was made between the fertility of daphnia grown on medium prepared from this extracted, finely powdered Wizard

*No visible ovarian development = —; oöcytes visible = +; green color in ovaries = ++.

**Ovarian regression.

and the fertility of daphnia where adequate vitamin E was added in the form of Triticol (4 drops per gallon). From an extracted finely powdered Wizard culture, containing twenty-three medium size nine-day-old daphnia, thirteen animals were placed in a museum jar containing a fresh supply of the same medium, while ten animals were similarly placed in a museum jar containing the vitamin E rich medium. The following records show a significant difference between the two groups.

TABLE IV

EFFECT OF VITAMIN E IN TRITICOL UPON 9-DAY OLD DAPHNIA GROWN IN PETROLEUM ETHER EXTRACTED, FINELY POWDERED WIZARD SHEEP MANURE

9-day old Daphnia used	Finely Powdered Wizard Extracted medium with Triticol	Finely Powdered Wizard Extracted Medium without Triticol
Start	10—*	13—
2 days	profuse internal fat-like storage	none
3 days	3—, 7+	10—, 3+
4 days	6+, 4++	4—, 9+
5 days	1+, 6++, 3 gravid	7—, 1+, 3++, 2 dead
6 days	1+, 4++, 4 gravid, 1 dead	4—, 3+, 3++, 1 dead
7 days	5++, 4 gravid	3—, 3+, 2++, 1 gravid
10 days	9 gravid, 14 young, excellent culture, observations discontinued	3—, 4+, 2 gravid, 1 young
17 days		7—, 1+, 1 dead, 1 young, 1+ and young removed and 25 mg. of Kieselguhr, saturated with Triticol added
19 days		4+, 3++
22 days		7 gravid
26 days		7 gravid, 20 young animals born between 22-26 days. Average of embryos in brood sac = 6

*No visible ovarian development = —; oöcytes visible = +; green color in ovaries = ++.

Similar results were obtained by using one-day-old animals instead of nine-day-old daphnia, but the beneficial action of the vitamin E rich medium is much more striking as indicated in Table V.

TABLE V

EFFECT OF VITAMIN E IN TRITICOL UPON 1-DAY OLD DAPHNIA GROWN IN PETROLEUM ETHER EXTRACTED, FINELY POWDERED WIZARD SHEEP MANURE

1-day old Daphnia	Finely Powdered Wizard Extracted Medium with Triticol	Finely Powdered Wizard Extracted Medium without Triticol
Start		
7 days	6++, 1 gravid; twice as large as those without Triticol	2—*, 2+, 1++
11 days	4++, 3 gravid, 11 young	2+, 1++, 2 gravid
16 days	2—, 2+, 2++, 2 gravid (many) 31 young	5 gravid (2, 2, 2, 1, 1, embryos) 8 young
	The average of young per daphnia = 7	The average of young per daphnia = 1.6

*No visible ovarian development = —; oöcytes visible = +; green color in ovaries = ++.

Detection of Excess Vitamin E

In order to see whether the presence of excess vitamin E could be shown in a culture medium where adequate fertility factors are present as in Bovung cultures, one day old daphnia, from the same culture as used in the preceding experiment, were placed in museum jars with regular Bovung, and Bovung to which 25 mg. of Triticol saturated Kieselguhr were added. The volume of culture medium was eight ounces in both instances. The effect of excess vitamin E can be seen in the increased number of young in the first clutch.

TABLE VI

DETECTION OF EXCESS VITAMIN E BY INCREASED NUMBER OF YOUNG

1-day old Daphnia	Bovung + Triticol	Bovung Regular
Start	6 daphnia	5 daphnia
5 days	1+*, 5 gravid	1+, 4++
7 days**	1++, 5 gravid	2++, 3 gravid
10 days	young per clutch, 20, 20, 20, 16, 14, 14	(1 gravid daphnia killed accidently) young per clutch 10, 10, 13
11 days	—	10
	Ave./1st clutch = 17.3	Ave./1st clutch = 11

V. DISCUSSION

The demonstrated positive response to vitamin E of daphnia, whose rhythmic ovarian function and reproduction had been experimentally inhibited, has an important bearing upon the understanding of the nutritional requirements of invertebrates.

According to Hodge, 4th (4), as early as 1916, Loeb and Northrop (6) concluded that some insects require accessory food substances analogous to, though "specifically different from that needed for pigeons, rats, and other warm-blooded animals." The conclusion was reached after they showed the nutritional dependence of *Drosophila* upon yeast. However, Bacot and Harden (1) in 1922, reported the two vitamins A and B, as understood in the study of vertebrate nutrition, are indispensible to *Drosophila* and that vitamin C is not necessary. While in the nutrition of *Tribolium confusum* Duv. (Coleoptera) Sweetman and Palmes (8) found a probable need for vitamin B, though no absolutely demonstrable need for vitamin A could be found. The requirement of vitamin A, in addition to vitamin B, as reported by Richardson (7) for the growth of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera) is held to be inconclusive. Zabinski (14) reported that the

*Oöcytes visible = +; green color in ovaries = ++.
**Transferred to individual bottles.

roach *Periplanata orientalis* (Orthoptera) is unable to mature on synethetic diets without vitamins.

Hodge, 4th (4) studied certain satisfactory and unsatisfactory diets as related to the nutritional requirements of *Melanoplus differentialis* Thomas (Orthoptera). Restricted diets led relatively to poorer development than shown by grasshoppers reared on non-restricted diets. In addition, growth was irregular, susceptibility to pathological conditions was present, and a higher mortality rate was recorded. Hodge, 4th (5) was able to correlate certain changes in the gastric epithelial cells of *Melanoplus differentialis* reared on restricted and non-restricted diets.

It is readily apparent from the literature cited that for certain classes of the Arthropoda, especially Insecta, the need for certain vitamins has been shown. The dependence of *Daphnia magna,* (Crustacea) on vitamin E, as clearly shown by the preliminary results obtained, adds another reported vitamin requirement for the invertebrates. At present, the investigation of the responses of the *Daphnia magna* to vitamin A, as possibly related to vitamin E, upon ovarian rhythm and reproduction is under way. In addition, studies on the testicular responses of male daphnia to vitamin E deficiency have been initiated.

The ovarian regression, reabsorption of oöcytes, seen in daphnia reared in vitamin E deficient culture media, suggests a possible analogy with the reabsorption of young in utero in rats maintained under vitamin E deficiency (15).

The possible use of *Daphnia magna* as a test animal in the quantitative estimation of the potency of synthetically prepared vitamin E (α-tocopherol) (2) is enhanced by the speed and simplicity of ovarian rhythm reproduction, thus permitting definite results within a few days.

SUMMARY AND CONCLUSIONS

1. *Positive Response of Daphnia magna to Vitamin E*

Daphnia magna, the transparent crustacean and Biological Reagent, responded to the presence and absence of vitamin E (the fertility vitamin), and ultimately might be used satisfactorily as an ideal test animal for the rapid detection and evaluation of vitamin E.

2. *Specific Symptoms of Vitamin E Deficiency and Recovery*

Daphnia, grown in vitamin E deficient culture medium which was made from petroleum ether extracted Wizard sheep manure, were inhibited in their growth, rhythmic ovarian function and reproduction. Moreover, there was a decided lack of vigor and their mortality rate was high. The addition of vitamin E. in the form of Triticol (cold pressed wheat germ oil) to such deficient daphnia accelerated, within forty-eight hours, their growth, restored the ovarian rhythm, increased the number of young per clutch, improved their vigor and decreased the mortality rate.

3. *Fertility Deficiencies in Stagnated Cultures Remedied by Vitamin E*

The reproductive rate of the daphnia growing in stagnated Bovung (cow manure) cultures, is very much reduced. In some instances, close observation of such animals revealed that their ovaries would develop to the stage where the individual oöcytes were visible and then regress or become reabsorbed. Similarly ovarian regression was seen in some of the animals cultured in petroleum ether extracted Wizard sheep manure. The addition of vitamin E rich food restored the ovarian rhythm and increased the reproductive rate.

4. *Response of Normal Daphnia to Excess Vitamin E*

One-day-old daphnia responded positively to the addition of excess vitamin E (in the form of Triticol) under cultural conditions where adequate fertility factors were present. There was a significant increase in the number of young in the first clutch released as contrasted with those animals into whose adequate culture medium no addition of vitamin E was made.

5. *Relative Value of Culture Media*

Bovung (cow manure) medium is superior to Wizard (sheep manure) for the propagation of Daphnia, while Wizard is superior to Michell sheep manure. The value of Cellu soy bean flour for the propagation of Daphnia has not been diminished by the results of this investigation. A supposedly vitamin E-free mix, designed for rat experiments, yielded variable results when tested on Daphnia, and no conclusion could be reached as to its absolute freedom from vitamin E.

6. *Outlook*

The results from these experiments are being extended to the quantitative study of the various tocopherols, and similarly the effect of other vitamins upon *Daphnia magna* should be investigated.

VII. BIBLIOGRAPHY

(1) Bacot, A. W., and Harden, A.: Vitamin Requirements of Drosophila I. Vitamins B and C. *Biochem. Jour. 16,* 148 (1922).

(2) Evans, H. M., Emerson, O. H., and Emerson, G. A.: Isolation from Wheat Germ Oil of an Alcohol, *a*-Tocopherol, Having the Properties of Vitamin E. *Jour. Biol. Chem. 113,* 319 (1936).

(3) Galtsoff, Paul (Editor): Culture Methods for Invertebrate Animals. Comstock Publishing Co., Ithaca, N. Y. (1937).

(4) Hodge, 4th, Charles: Growth and Nutrition of *Melanoplus differentialis,* Thomas (Orthoptera: Acrididae) I. Growth on a Satisfactory Mixed Diet and on Diets of Single Food Plants. *Physiol. Zool. 6,* 306 (1933).

(5) Hodge, 4th, Charles: Some Effects of Diet on the Gastric Epithelial Cells of the Grasshopper (*Melanoplus differentialis,* Thomas). *Biol. Bull. 72,* 203 (1937).

(6) Loeb, J., and Northrop, J. H.: Nutrition and Evolution. *Jour Biol. Chem. 27,* 309 (1916).

(7) Richardson, C. H.: Physiological Study of the Growth of the Mediterranean Flour Moth (*Ephestia kuehniella,* Zell.) in Wheat Flour. *Jour. Ag. Res. 32,* 895 (1926).

(8) Sweetman, M. D., and Palmer, L. S.: Insects as Test Animals in Vitamin Research I. Vitamin Requirements of *Tribolium confusum,* Duvall. *Jour Biol. Chem. 77,* 33 (1928).

(9) Viehoever, Arno: Daphnia Propagation for Experimental Use. *A. J. Ph. 107,* 3 (1935).

(10) Viehoever, Arno, and Cohen, Isadore: Mechanism of Strychnine Action I. The Physiological Evaluation. *A. J. Ph. 109,* 285 (1937).

(11) Viehoever, Arno: Report on Rhubarb and Rhaponticum, II. *Jour. O. A. A. C. 20,* 562 (1937).

(12) Viehoever, Arno: Field Tests for Marihuana (Cannabis). *A. J. Ph. 109,* 12 (1937).

(13) Viehoever, Arno, and Cohen, Isadore: Mechanism of Action of Aphrodisiac and Other Irritant Drugs, I. Physiological Evaluation of Yohimbine, Cantharidin, Capsaicin and Piperine on Daphnia Magna. *A. J. Ph. 110,* 6 (1938).

(14) Zabinski, J.: Elevage des blattides soumis à une alimentation artificielle. *Compt. Rend. Soc. de Biol. 98,* 73 (1928).

(15) Zagami, V., and Sindomi, M.: Pregnancy and Intra-Uterine Histologic Changes in Rats Kept on a Diet Lacking Vitamin E. Death and Reabsorption of Fetus. *Riv. di pat. sper. 12,* 1 (1934).

ERRORS IN THE USE OF STANDARD THIOSULFATE SOLUTIONS CONTAINING SODIUM TETRABORATE PRESERVATIVE

By C. W. Jordan

THE most important factor in iodometry is the reaction between iodine and sodium thiosulfate. It is, therefore, prerequisite that the standard solutions used should be permanent and easily reproducible.

Properly made 0.1 N. iodine solution kept in a well-stoppered brown bottle in a cool place will retain its titer over a long period of time. On the other hand, standard sodium thiosulfate solutions often decrease in titer after preparation. This appears to be particularly true of 1/100th and lower normality solutions.

Various theories have been advanced to account for this loss in titer and an excellent summary of the published work on the subject is given by Kolthoff.[1]

Among the explanations given were, decomposition by carbonic acid, oxidation by air with the intermediate formation of sulfate, catalytic decomposition due to traces of copper in the water used, and photogenic decomposition.

Kolthoff [2] tried the addition of many substances to thio solutions for preserving the titer, including borax, sodium carbonate, sodium bicarbonate, sodium benzoate, sodium fluoride, copper sulfate, formalin, silver nitrate, phenol, petroleum ether, sodium phosphate, ferrous sulfate, sodium sulfite, and sodium hydroxide. The most efficient appeared to be 500 mgs. per liter of either sodium carbonate or sodium hydroxide.

The work of Mayr [3] and Mayr and Kerschbaum [4] indicated that decomposition of thiosulfate solutions was due solely to micro-organisms and could be prevented by the addition of bactericides or growth inhibitors.

The bacteria thought to be mainly responsible were the thiosulfate bacteria first found in sea water by Nathansohn [5] and later found by Klein and Limberger [6] to exist in the air. The decomposition of thiosulfate solutions proceeds in two steps:

$$\text{(a)}\quad Na_2S_2O_3 \rightarrow Na_2SO_3 + S$$
$$\text{(b)}\quad Na_2SO_3 + O \rightarrow Na_2SO_4$$

The most promising bactericide was mercuric iodide (10 mgs. HgI_2 per liter of N/100 $Na_2S_2O_3$ solution) but the thiosulfate solution was found to become turbid upon long standing and a deposit of mercuric sulfide formed in the bottom of the container. Other substances acting as efficient bactericides were 1 per cent. by volume of amyl alcohol and 5 per cent. by volume of ethyl alcohol.

The addition of compounds having alkaline reaction, principally ammonium carbonate, sodium carbonate and sodium hydroxide, had long been recommended for preventing decomposition of thiosulfate solutions. Mayr and Kerschbaum [4] investigated the various strengths of sodium carbonate recommended from the standpoint of inhibiting bacterial action. They worked with N/100 thiosulfate solution, to which sodium carbonate was added in amounts to vary the alkalinity normality between N/200 and N/25,000.

They concluded that the addition of sodium carbonate to sterile thiosulfate solutions was unnecessary as decomposition never occurred and that the most efficient concentration to use in infected solutions was between N/1,000 and N/2,000. This fixed the optimum pH for inhibiting bacterial action between 9.0 and 10.0. Further investigation showed that the addition of 3.8 gms. of crystallized borax (1/100th molar solution) per liter of N/100 thiosulfate solution, giving a pH of 9.18 was more effective than the optimum concentration of sodium carbonate, in fact, it was about equal to the bactericide mercuric iodide after 90 days test.

The use of borax as a preservative of very dilute thiosulfate solutions has been quite generally recommended since the publication of the work of Mayr and Kerschbaum. It has also been recommended for use in N/10 solutions despite the fact that they are much less subject to decomposition, relatively, than weaker solutions.[7]

Errors in the Use of Alkaline Thiosulfate Solutions

In standardizing thiosulfate solutions containing alkaline preservatives against neutral solutions containing known weights of dissolved iodine serious errors may be incurred due apparently to the oxidation of part of the thiosulfate to sulfate in the presence of hydroxyl ions. The oxidation of thiosulfate solutions was first described by Topf,[8] and later investigations were made by Taylor,[9] Puckner [10] and Battey.[11]

The reaction is assumed to proceed in two stages, first, the formation of hypoiodite followed by oxidation of the thiosulfate.

(1) $8\,NaOH + 4\,I_2 \rightleftarrows 4\,NaOI + 4\,NaI + 4\,H_2O$

(2) $Na_2S_2O_3 + 4\,NaOI + 2\,NaOH \rightleftarrows 2\,Na_2SO_4 + 4\,NaI + H_2O$

The complete reaction may be represented by the following ionic equation:

$$S_2O_3^{=} + 4\,I_2 + 10\,OH^{-} \rightarrow 2\,SO_4^{=} + 8\,I^{-} + 5\,H_2O$$

It is evident from the stoichiometric relations that in the standardization of thiosulfate solutions the amount required to combine with a given weight of iodine in the presence of alkali will be considerably reduced.

Kolthoff [2] pointed out possible errors from this source in titrating 0.1 N. iodine solutions to which various amounts of sodium bicarbonate, sodium phosphate (monobasic), sodium tetraborate and ammonium carbonate were added. The greatest reduction in the amount of thiosulfate required was in the case of ammonium carbonate (10 ml. of 2 N. ammonium carbonate added to 25 ml. of 0.1 N. iodine) where an error of —38.0 per cent. was noted.

Experiments performed by the writer showed that in the standardization of 0.1018 N. thiosulfate solution containing 3.8 G. P. L. of $Na_2B_4O_7\,10\,H_2O$ (standardization of thiosulfate solution with and without added borax by the potassium dichromate method in acid solution gave identical values) by titrating about 0.5 gm. of dissolved iodine according to the procedure described by Treadwell [12] gave an indicated normality of 0.1059 or 4.0 per cent. too high.

This error in indicated normality is not constant but appears to vary with the rate of addition of alkaline thiosulfate to the iodine solution, the hydroxyl-ion concentration, the concentration of iodine and the temperature.

The error may be accentuated as shown by the following experiments: Neutral sodium thiosulfate solution having a true normality of 0.10498 was used in titrating known weights of iodine in solution containing (1) no borax, (2) 100 ml. of 1/100th molar solution of $Na_2B_4O_7\,10\,H_2O$ and (3) the same quantity of borax as (2) but neutralized by H_2SO_4 using methyl red indicator prior to adding the iodine.

The results obtained are shown in the following table:

TABLE I

EFFECT OF SODIUM TETRABORATE IN IODINE SOLUTION ON THE STANDARDIZATION OF 0.1 N. SODIUM THIOSULFATE

	Test #1	Test #2	Test #3
gms. Iodine used	.5796	.5070	.5463
gms. KI used	3.0	3.0	3.0
ml. Water	100.0	100.0	100.0
ml. 1/100th molar solution $Na_2B_4O_7$ $10H_2O$	0	100.0	100.0 (neutralized)
ml. thiosulfate required	43.5	35.0	41.0
Apparent normality of thiosulfate	.10498	.11413	.10498

The apparent normality as determined by test #2 in the presence of borax was 8.7 per cent. higher than the true value indicated by test #1. Test #3 shows that the presence of boric acid was without influence on the determination of normality of the thiosulfate.

Bottomley [13] described experiments on reactions of iodine with water solutions of borax. He inferred that borax hydrolyzed in solution according to the reactions:

$$Na_2B_4O_7 + 3\,H_2O \rightleftarrows 2\,NaBO_2 + 2\,H_3BO_3$$
$$NaBO_2 + 2\,H_2O \rightleftarrows NaOH + H_3BO_3$$

Iodine was then said to react with the dilute sodium hydroxide solution to form sodium iodate and sodium iodide:

$$3\,I_2 + 6\,NaOH = 5\,NaI + NaIO_3 + 3\,H_2O$$

However, these reactions are not believed to occur under conditions existing in the standardization of thiosulfate solutions with a solution of a known weight of iodine since potassium iodate was never found in the solutions after titration with thiosulfate containing borax.

In titrating alkaline thiosulfate solutions with iodine solutions less error is introduced than in the reverse titration since iodine reacts rapidly and selectively with the thio instead of the hydroxyl-ions and hence less hypo-iodous acid is formed. This is illustrated by the following tests:

TABLE II

TITRATION OF IODINE SOLUTION WITH THIO SOLUTIONS (a) NEUTRAL, AND (b) CONTAINING 3.8 G. P. L. OF BORAX

Ml. .09976 N. Iodine	(a) Required Ml. Thio without Borax	(b) Required Ml. Thio with Borax	Apparent Normality of Thio
50.0	49.88		0.1000
50.0		47.44	0.1051

TABLE III

TITRATION OF THIO SOLUTIONS (a) NEUTRAL, AND (b) CONTAINING 3.8 G. P. L. OF BORAX, WITH IODINE SOLUTION

(a) Ml. Thio without Borax	(b) Ml. Thio with Borax	Ml. .09976 N. Iodine	Apparent Normality of Thio
50.0		50.12	.1000
	50.0	51.27	.1023

Errors in the Use of Alkaline Thiosulfate Solutions with the McIlhiney Bromine Method of Determining Organic Unsaturation

The use of alkaline thiosulfate solution in the McIlhiney [14] bromine method of determining organic unsaturation is to be avoided as the two-fold error involved is excessive. Briefly, this method of analysis consists in adding an excess of bromine solution to the diluted sample under test and the unconsumed bromine determined by adding potassium iodide solution and titrating the iodine liberated with standard thio solution. Any HBr formed by reason of substitution occurring is then determined by adding potassium iodate to the mixture and again titrating the iodine liberated with standard thio solution.

The residual alkalinity of the thio solution used in the first part of the titration is often sufficient to neutralize the entire quantity of HBr present and hence no reaction is obtained upon adding KIO_3 solution.

This results in an erroneous and abnormally high addition bromine value which in some cases was found in error about 50 per cent.

SUMMARY

(1) The history of the use of borax and other substances as preservatives of standard thiosulfate solutions is given.

(2) Errors in the use of alkaline thiosulfate solutions are described.

(3) The use of alkaline thiosulfate solutions in the McIlhiney bromine method of determining organic unsaturation is to be particularly avoided since a two-fold error is involved.

LITERATURE CITED

1. Kolthoff, I. M.: "Volumetric Analysis," Vol. I (1928). Translation by N. H. Furman, pp. 231-234.

2. Kolthoff, I. M.: *Zeitschrift für Analytische Chemie, 60,* 338 (1921).

3. Mayr, C.: *Ibid., 68,* 274 (1926).

4. Mayr, C., and Kerschbaum, E.: *Ibid., 73,* 321 (1928).

5. Nathansohn, A.: *Mitt. a. d. Zoolog. Station Neapel, 15,* 658 (1902).

6. Klein, G., and Limberger, A.: *Biochem. Zeitschr., 143,* 473 (1923).

7. Watson, F. J.: *Soc. Chem. Ind. Victoria Proc., 32,* 679-680 (1932); Yoshida, I: *J. Chem. Soc. Japan, 48,* 26-27 (1927); Pierce and Haenisch: "Quantitative Analyses" (1937), p. 179; Hall. W. T.: "Textbook of Quantitative Analysis" (1935), p. 117; Treadwell-Hall: "Analytical Chemistry," Vol. II, "Quatitative Analysis" (1935), p. 598; Biltz, H., and Biltz, W.: "Ausführung Quantitativer Analysen" (1937), p. 131.

8. Topf, G.: *Zeit. Analyt. Chem., 26,* 137 (1887).

9. Taylor, R. L.: *J. Chem. Soc., 77,* 725 (1900).

10. Puckner, W. A.: *American Pharmaceutical Association Proceedings, 52,* 408 (1904).

11. Battey, J. P.: *Analyst, 36,* 132 (1911).

12. Treadwell-Hall: "Analytical Chemistry," Vol. II, "Quantitative Analysis" (1935).

13. Bottomley, J.: *Chemical News, 31,* 76 (1875).

14. McIlhiney, P.: *J. Am. Chem. Soc., 16,* 275 (1894); *J. Am. Chem. Soc., 21,* 1084 (1899); *J. Am. Chem. Soc., 24,* 1109 (1902).

THE PHILADELPHIA » » COLLEGE OF PHARMACY AND SCIENCE » » »

THE ONE HUNDRED AND SIXTEENTH ANNUAL COMMENCEMENT

THE One Hundred and Sixteenth Annual Commencement of the Philadelphia College of Pharmacy and Science was held in the College auditorium evening of June 8th, in the presence of a large audience. The invocation was pronounced by the Reverend John Schmidt, of Union City, N. J., father of Arthur M. Schmidt, a member of the graduating class in pharmacy.

Candidates for degrees were presented to President Wilmer Krusen by Deans Ivor Griffith and J. W. Sturmer. Degrees in course in pharmacy, chemistry, bacteriology and biology were conferred upon 92 students.

The degree of Doctor of Pharmacy, honoris causa, was received by:

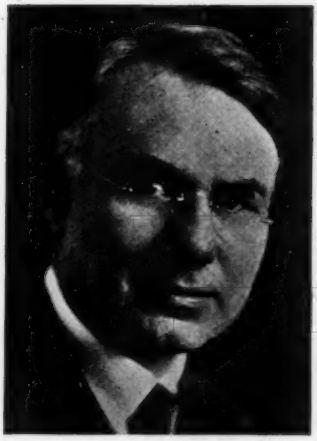
George W. Merck, president of Merck & Company, Rahway, N. J.

Dr. Arno Viehoever, research professor of biology and biochemistry at this College.

The degree of Master of Pharmacy, honoris causa, was received by:

Dr. Marvin R. Thompson, professor of pharmacology in the school of pharmacy at the University of Maryland.

Dean Hugh C. Muldoon, dean of Duquesne University College of Pharmacy, and president of the American Association of Colleges of Pharmacy, prepared the commencement address, but in his absence, due to serious illness, the address was read by his colleague, Professor Earl Guth.



DR. ARNO VIEHOEVER

DR. MARVIN R. THOMPSON

DR. GEORGE W. MERCK

Degrees in course, certificates and prizes were conferred as follows:

DOCTOR OF SCIENCE IN CHEMISTRY
William G. Batt

DOCTOR OF SCIENCE IN PHARMACY
Chimanlal B. Shah

DOCTOR OF SCIENCE IN BACTERIOLOGY
George M. Eisenberg

MASTER OF SCIENCE IN CHEMISTRY
Elliott E. Leuallen

MASTER OF SCIENCE IN PHARMACY

John P. Barlement
John L. Hoffman, 2d
Madeline O. Holland
Edward F. Merdinyan

MASTER OF SCIENCE IN BACTERIOLOGY

Bernard Witlin

BACHELOR OF SCIENCE IN CHEMISTRY

John R. Cox
Richard D. Fine, III
Jonathan H. Lengel
James W. Mitchell
Joseph M. Perri
Frederick W. Schiller
Kenneth E. Shull
William B. Spear
George B. Wellburn
Robert E. Wolfrom
Harry C. Zeisig, Jr.

BACHELOR OF SCIENCE IN BACTERIOLOGY

Frances J. Finnigan
Helen E. Gaskill
Eleanor Goldfarb
Sylvia King
Milton L. Lewis
David Tomkin
Jorge E. Zepeda

BACHELOR OF SCIENCE IN BIOLOGY

Edmund H. MacLaughlin

BACHELOR OF SCIENCE IN PHARMACY

Dorothy F. Albino
Ralph M. Belinsky
Norman E. Benner
Harry Bernstein
Albert L. Berrettini
Adolph F. Borkowski
Herman Braman
Jack W. Brodman
Rose Capobianco
Louis J. Caruso
Joseph L. Ciminera
Francis A. Cook
Kenneth R. Crispell
Felice J. DeMaria
Michael P. DeVittorio
Warren E. Dickinson
Edward F. Dougherty
James A. Doyle, Jr.
Dominic Figlio
Leonard S. Forman
Martin J. Gardner
Franklin Gasser
Benjamin Greenbaum
Eugene Heller
Manuel A. Johnson, Jr.
Carl A. Kinsey
Dorothy I. Kleckner
Raymond Klevansky
William G. Knapp, Jr.
Martin J. Laskin
Leonard J. Loiacono
Leo L. Lucci
Raymond J. McGinnis
Robert L. McNeil, Jr.
Warren R. McPeek
William G. Marsh
Pauline M. Melcher
Elmer W. Merz
Robley H. Millard
Grayson A. Moore
John J. Moran
Anthony D. Nedzinski
Henry J. Pasquarello
Carlo A. Persichetti
Irving Porten
Ralph M. Rapp
Henry L. Ritter
Aaron Robkin
Carl A. Ruggier
Chester J. Sasadeusz

Gerald S. Savitz
Arthur M. Schmidt
Joseph N. Sedor
Edward J. Solvibile
William A. Thawley
Charles O. Truxton
Daniel Ungar
Gerard H. Warner
Victor Weiss
John M. Woodside, Jr.
Edwin Zaslov
Edwin K. Zechman
Nathan Ziskin
Solomon Zoltick

Harry L. Zwald

CERTIFICATES IN CLINICAL CHEMISTRY

Vito Esposito
Abrahams Bazrod
Max Jaffe

CERTIFICATES IN BACTERIOLOGY

Max Jaffe
Abrahams Bazrod
Betty F. Kessler

AWARD OF PRIZES

Designated as "Distinguished"
With General Average Over 90%

Joseph L. Ciminera
Felice J. DeMaria
Robert L. McNeil, Jr.
Robley H. Millard
John M. Woodside, Jr.
James W. Mitchell

Kenneth E. Shull

Designated as "Meritorious"
With General Average Between 85% and 90%

Dorothy F. Albino
Ralph M. Belinsky
Albert L. Berrettini
Herman Braman
Jack W. Brodman
Francis R. Crispell
Martin J. Gardner
Franklin Gasser
Eugene Heller
William G. Knapp, Jr.
Leonard J. Loiacono
Elmer W. Merz
Carlo A. Persichetti
William A. Thawley
Charles O. Truxton
Edwin Zaslov
John R. Cox
Jonathan H. Lengel
Joseph M. Perri
Frederick W. Schiller
George B. Wellburn
Harry C. Zeisig

The PROCTER PRIZE, a gold medal awarded to the B. Sc. candidate in Pharmacy having the highest average of the class. Earned by:

JOSEPH L. CIMINERA

Honorable Mention to

Felice J. DeMaria
Robley H. Millard
Robert L. McNeil, Jr.
John M. Woodside, Jr.

The FRANK GIBBS RYAN PRIZE, a gold medal endowed by the Class of 1884, as a memorial to their distinguished classmate, for the best average in the Chemical and Pharmaceutical Laboratory Courses, is awarded to:

JOSEPH L. CIMINERA

Honorable Mention to

Felice J. DeMaria
Franklin Gasser
Martin J. Gardner
John M. Woodside, Jr.

The WILLIAM B. WEBB MEMORIAL PRIZE, twenty dollars and a bronze medal for the highest average in the branches of Operative Pharmacy, Analytical Chemistry, and Pharmacognosy, is awarded to:

JOSEPH L. CIMINERA

Honorable Mention to

Felice J. DeMaria
Carlo A. Persichetti
Robley H. Millard
John M. Woodside, Jr.

The FREDERICK WILLIAM HAUSSMANN MEMORIAL PRIZE of one hundred dollars, given to the Pharmacy student with the highest average for the last three years of the course, is awarded to:

JOSEPH L. CIMINERA

Honorable Mention to

Felice J. DeMaria
Robley H. Millard
John M. Woodside, Jr.

A prize of twenty-five dollars offered by THE WOMEN'S AUXILIARY OF THE DAUPHIN, CUMBERLAND, AND LEBANON COUNTIES PHARMACEUTICAL ASSOCIATION to the girl graduating with the hightest average:

DOROTHY F. ALBINO

Gold Medals awarded by the Alumni Association to the student of the B. Sc. Class in Pharmacy and to the student of the B. Sc. Class in Chemistry, in Bacteriology, or in Biology who attain the highest scholastic averages, are awarded to:

B. Sc. in Pharmacy ..... JOSEPH L. CIMINERA
B. Sc. in Chemistry ..... JAMES W. MITCHELL

The REMINGTON MEMORIAL PRIZE, twenty dollars, offered by the Estate of Joseph P. Remington, for the highest average in the examination of Operative Pharmacy and Dispensing, is awarded to:

ROBERT L. MCNEIL, JR.

Honorable Mention to

Joseph L. Ciminera John M. Woodside, Jr.

The MAHLON N. KLINE THEORETICAL PHARMACY PRIZE, fifty dollars in cash, offered by the Mahlon N. Kline Estate, for the highest average in Theory and Practice of Pharmacy, is awarded to:

FELICE J. DEMARIA

Honorable Mention to

Joseph L. Ciminera Robert L. McNeil, Jr.
Martin J. Gardner Elmer W. Merz
John M. Woodside, Jr.

The MAISCH BOTANY PRIZE, a special prize of twenty dollars, offered by Sinclair S. Jacobs of the Class of 1909 to the member of the graduating class who shall have presented the best herbarium collection of plants, or the best thesis on the microscopical structure of medicinal plants, is equally divided between:

FELICE J. DEMARIA RALPH M. BELINSKY

The ALPHA SIGMA PRIZE is awarded to:

KENNETH E. SHULL

Honorable Mention to

James W. Mitchell

The AMERICAN INSTITUTE OF CHEMISTS' AWARD to:

KENNETH E. SHULL

ABSTRACTS FROM AND REVIEWS OF THE LITERATURE OF THE SCIENCES SUPPORTING PUBLIC HEALTH

Bacteriology . . . . Louis Gershenfeld, B. Sc., Ph M.
Biochemistry, Nutrition, etc. . . . Arno Viehoever, Ph. D.
Biology . . . . . Marin S. Dunn, Ph. D.
Chemistry . . . . . Arthur Osol, Ph. D.
Pharmacy . . . . . E. Fullerton Cook, Ph. M.
and their assistants

The Determination of Sulfanilamide. E. K. Marshall, Jr., and J. T. Litchfield, jr. *Science 88,* 85 (1938). The method for the determination of sulfanilamide in blood and urine as proposed by Marshall (*J. A. M. A. 108,* 953 (1938)) has been widely used both in experimental work and in controlling the administration of the drug to patients. This article calls attention to certain improvements in the method. By destroying the excess of nitrous acid after diazotization and buffering the solution before coupling with the dimethyl-α-naphthylamine more rapid color development and more permanent colors are obtained. In addition, it has been found that in the presence of sodium chloride and certain other substances the excess nitrous acid destroys some of the azo dye formed. This is entirely avoided by destruction of the excess nitrous acid after diazotization is complete.

The revised method is published in detail for those who may have occasion to conduct such a determination. L. F. T.

Inhibition of the Benzidine Blood Test by Ascorbic Acid. R. Kohn and R. M. Watrous. *J. Biol. Chem. 124,* 163 (1938). It has been found that the urine of experimental animals receiving large amounts of ascorbic acid failed to give a positive benzidine test for blood even when blood was known to be present in considerable amounts. The authors have investigated the clinical significance of this observation. There have been several reports in the literature concerning substances which may give a false positive test but very

little may be found relative to the possible inhibition of the test. Tauber (*Enzymologia 1, 209* (1936)) showed that peroxide-peroxidase oxidizes ascorbic acid very rapidly if substances capable of forming quinones are present. The quinones are reduced by ascorbic acid and in turn are reoxidized by the peroxide-peroxidase system, the reaction continuing until either all the ascorbic acid or all the peroxide is exhausted. This paper did not deal with the benzidine test, although benzidine falls in the class of quinone-forming compounds. The present authors have investigated carefully the effect of ascorbic acid on the benzidine test with the following interesting results. The mechanism of interference was found to be the reduction by the ascorbic acid of the blue compound usually formed in a positive test to a colorless state. In fact if ascorbic acid is added to a test already showing a strongly positive reaction the mixture quickly becomes colorless. Ascorbic acid which has been oxidized with permanganate until it gives no further evidence of reducing power by the iodine titration method is still able to interfere with and reverse the benzidine test but in a weaker manner. As would be expected the intensity of any benzidine reaction to blood in the presence of ascorbic acid may be stated roughly to be directly proportional to the concentration of hemoglobin and inversely proportional to the concentration of ascorbic acid. The interference of ascorbic acid with the benzidine test may, for ordinary clinical work, be avoided by performing the test only on the ether extract of acidified urine as recommended by Hawk and Bergeim. L. F. T.

Trophophylactic Power of Certain Edible Oils Toward Toxic Substances. P. Lassabliere, M. Uzan and A. Monnet. *Compt. rend, 206,* 1592 (1938) through *Squibb Abstr. Bull. 11,* 1150 (1938). The subcutaneous administration of 0.5 cc. of olive, peanut, palm, sesame or almond oil into mice one-half hour before the subcutaneous injection of an ordinarily lethal dose of sparteine sulfate, hydroalcoholic extract of amanita, cobra venom, or mercury cyanide permitted 81 of 84 animals to survive longer than controls not given the oils before the toxins. Death did not occur in 38 per cent. of the oil-treated animals. The "trophophylactins," i. e., the agents responsible for this "trophophylactic" effect cannot be vitamins since the oils used contained little or no vitamins. L. F. T.

The Detection and Colorimetric Determination of Parahydroxybenzoic Acid Esters in Food and Similar Products. Th. Sabalitischka. *Micro-Chem. Acta 2,* 111 through *Pharm. Zentrh. 79,* 400 (1938). The author discusses first the isolation of the ester in the experimental material: (1) Fat-free liquid material. 5 grams of substance, weighed accurately to 0.1 gram, are mixed in a separator with 15 cc. of water and 2 cc. of 25 per cent. sulfuric acid, and washed out twice with 25 cc. ether. The ether is evaporated in a flask, dissolved in two 2 cc. portions of boiling water and made up to 5 cc. The solution serves for a qualitative detection or for a colorimetric determination. (2) Fat-free, more or less solid material. In a reflex condenser (or Soxhlet) one extracts 5 grams of substance (prepared as above) three times with 25 cc. portions of ether. Proceed as above. (3) Fatty material. 5.0 grams of substance is weighed accurately to 1.0 gram and dissolved in 50 cc. of ether in a short stemmed separatory funnel. The weighing bottle is washed out with 20 cc. and 15 cc. portions of 5 per cent. potassium hydroxide solution which are added to the separator. The separator is then thoroughly shaken. After separation of the two layers one draws off the alkaline solution and acidulates with 15 cc. of 25 per cent. sulfuric acid. If the solution is turbid, one mixes it with 6 drops of saturated zinc sulfate solution and then with 2 drops of potassium ferrocyanide solution, filtered (using kieselguhr) and washed with water. Zinc sulfate must be present in excess. The now clear acid solution is shaken out twice with half its volume of ether and treated again as in (1). When a difficultly separable emulsion is encountered, one saponifies the ester by warming the ester-containing layer fifteen minutes on a water bath and proceeds again as above, but must shake out four times with ether in order to obtain all the free hydroxybenzoic acid. (4) Emulsified material is broken down by boiling with ten times the volume of water and 5 cc. sulfuric acid. After the cold solution is cleared up with zinc sulfate and potassium ferrocyanide and filtered through kieselguhr as before, the residue (fat) is washed out with 25 cc. of ether and the filtrate shaken out with ether. The same operation is repeated once again, the ether fraction being subsequently treated with alkali as in (3).

Qualitative and Quantitative Determination of the Ester

The author detected as little as 0.001 mgm. of p-hydroxy-benzoic acid with Millon's reagent (1 part mercury dissolved in 1 part nitric

acid and diluted with 2 parts of water, then filtered). The retained residue from the ether shakeouts is dissolved in two 2 cc. portions of water, treated with 1 cc. of reagent, and heated for one minute in a boiling water bath. The color of the reaction is red and will partially disappear in an ether shakeout.

For the colorimetric determination it is necessary to warm the ester with the reagent in an incubator at 37° C. for one or two hours. After making up the residue to 5 cc. add exactly 0.05 cc. concentrated nitric acid and 0.5 cc. Millon's reagent (using a micro-pipette). Mix thoroughly after addition of each portion. After an hour at 37° C. one compares this solution with a standard solution of 0.1 per cent. Nipagin, made up in 10 dilutions from 0.01 to 0.1, which have been similarly treated with nitric acid and the reagent. R. L. M., JR.

Observation on Blood Sugar Level Before, During and After Hunger Periods in Humans. W. W. Scott, C. C. Scott and A. B. Luckhardt. *Amer. Jour. Physiol. 123,* 243 (1938). A considerable amount of work has been done in an attempt to determine any relationship between the blood sugar level and the cause of spontaneous gastric motility (hunger contractions). Earlier work indicated the possibility that the blood sugar level was inversely related to hunger contractions.

The authors used the conventional balloon-water manometer method to record the hunger contractions in healthy human subjects. At the same time, using the microchemical method of Miller and Van Slyke, blood sugar determinations were made preceding, during and after normal hunger periods (5-6 hours).

Within the range of experimental error (5 mgm. per cent.) no variations in the blood sugar level were found during the entire period. The level of blood sugar evidently bears no causal relation to the normal "hunger periods" in man. I. C.

The Effect of Alcohol on the Hunger Sense. C. C. Scott, W. W. Scott and A. B. Luckhardt. *Amer. Jour. Physiol. 123,* 248 (1938). The purpose of this research was to determine, more accu-

rately than previously reported by other workers, the exact nature of increasing the desire for food. Earlier conclusions seemed to indicate that alcohol stimulated the appetite while abolishing hunger.

The balloon-water manometer method was employed to record the hunger contractions in healthy human subjects. The dosage of alcohol was made to correspond to a cocktail or two, enough to produce a moderate feeling of exhilaration or headiness. It was found that 200 cc. of 20 per cent. alcohol in two 100 cc. doses, about five minutes apart, produced the desired reaction.

The results show that the hunger contractions occurring after the primary inhibitory effect due to the ingested alcohol, produced *hunger sensations* which were more intense, (even slight contractions producing hunger pangs) as compared with the inconsequential effect of similar contractions occurring in the control period. The alcohol did not increase the amplitude of the hunger contractions but instead produced an epigastric sensation during the period of alcohol inhibition preceding the hunger contractions. This feeling was associated with an increased desire for food, however, less intense than hunger but continuous; consequently, this feeling was considered to be in the nature of an appetite. I. C.

Analysis of Caramel Color. W. R. Fetzer. *J. Ind. & Eng. Chem. Anal. Ed. 30,* 349 (1938). Caramel color is very extensively used in various foodstuffs and drugs. Very little has been published concerning the testing of caramel. Probably the best article is that of Salamon and Goldie (*J. Soc. Chem. Ind. 19,* 301 (1900)). The tests proposed by these authors are largely in use today. Due to the wide variation in the procedures employed in the manufacture of caramel there is wide variation in the finished product. This has led to consumers of the product adhering closely to one source of supply once it has proven satisfactory for their own individual use.

The greatest difficulty in the analysis of caramel lies in the multitude of methods in use with the consequent lack of uniformity in evaluation by different laboratories. In addition, most laboratory analyses are not sufficiently comprehensive to cover caramel in general, but are built around a specific use. A caramel which may be well suited for one use is often quite unsuited for another. Thus any

consideration of caramel quality must take into consideration the service to which the caramel is to be put.

The author presents details of several tests which serve as a criterion of caramel quality, included are Specific Gravity, Tinctorial Power, pH, Viscosity, Ash, Iron. In addition, several special tests such as, Acid Test, Neutral Tannin Test, Fermentation Test, Foam Test and Compatibility are outlined which serve to evaluate a caramel to be used for some specific purpose. Typical results of analyses with their significance are included. L. F. T.

In response to a query from a practicing pharmacist, the Operative Pharmacy Department of the College has supplied the following information:

Urea is being extensively used in surgical technic in from 5 to 25 per cent. aqueous solution. In treating a wound the gauze dressing is kept moist continuously with the solution. The tendency recently has been to increase the strength and it is now believed that strengths even in excess of 25 per cent. are the most effective. One manufacturer has found that 0.5 per cent. of chlorobutanol is an entirely satisfactory preservative, keeping the solution clear and free from deterioration. In preparing it, it is best to dissolve the chlorobutanol first with the aid of heat and then allow the solution to cool before dissolving the urea. The latter is entirely soluble but dissolves very slowly. Heat, however, is reported to cause decomposition.

Nicotinic Acid is reported as being non-hygroscopic, non-deliquescent, and stable in air. Its melting point is 235° C., therefore it would seem as though it is entirely stable and as the average dose is approximately 0.5 Gm. per day when administered orally, this could be placed directly into capsules. One report recommends that the 0.5 Gm. daily should be administered in five 0.1 Gm. doses. However, this quantity can readily be placed in capsules without diluent. If a diluent is to be used, starch is suggested.

Vitamin B_1 (Thiamin Chloride, Aneurin), Vitamin B_2 or Vitamin G (Riboflavin)—These two products are stated to be quite stable, especially the vitamin B_2 which is capable of withstanding intensive heat treatment, even the temperature of the autoclave. It is suggested for these that a diluent of starch be used when administered in capsules.

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

Despite the form in which this information is presented it may be accepted as trustworthy and up-to-date. Original sources are not listed but they may be obtained upon request.

Catching fish for fun and for food is an ancient occupation, and devious are the means of their catching. Nets, hooks, spears, guns, poisons to say nothing of "smells" are the various methods whereby these denizens of the deep are corralled. But speaking of fishing with "smells" it is said that civet and musk are most attractive to certain fish and artificial bait so scented is alleged to double its attractiveness.

Fish have powerful "smellers." For instance you can hang out a thoroughly closed pail of minnows in your boat house, that is, if the boat house is in the right spot, and you will find the next morning pikes and black bass in the vicinity of the pail, although they certainly cannot see the fish inside the pail and it must therefore be the odor which attracts them.

And speaking of fishing, one instantly thinks of that pestilential foe of every local angler, namely the mischievous mosquito, whose bayonet thrusts yield mounds of itchy flesh wherever they happen to sting. Isaac Walton could never have written his calm, beguiling book had he fished in mosquito infested fish-haunts. But here is a formula for an application to exposed flesh, which is alleged to be externally deterrent to this pesky bug, and I have it on the authority of a Jerseyite, whose terrain is where the great mosquitoes grow each as large as a brace and bit,—I have it on his authority that they thoroughly dislike this combination which is curative as well as prophylactic.

Menthol	*20 grams*
Phenol	*30 grams*
Ethyl alcohol	*1 fluid ounce*
Diethyl phthalate	*1 fluid ounce*
Glycerin	*2 fluid drams*
Oil of citronella to make	*4 fluid ounces*

The solids are dissolved in the liquids. Application must shun the eyes and mucous tissues.

And we've called it the "weaker sex"!

But listen:

It was disclosed at the recent meeting of the National Association of Insecticide and Disinfectant Manufacturers that female flies can resist insecticides longer than male flies and that, therefore, the official Peet Grady test for measuring the killing power of fly sprays should be revised to take account of this difference.

For those who wish a large-scale differential test let a looking-glass be placed in the test room, the females will be collected around it—and the males will be buzzing around elsewhere!

Who said that there is no such thing as "perpetual motion." Think of this:—In sewage disposal systems the digestion of sewage solids by bacteria produces gas; this gas is utilized for producing hot water; this hot water is returned to the digester to raise the temperature to a point which will produce more rapid bacterial digestion; the more rapid digestion produces more gas which may in turn be used for the increasing of bacterial activity and the consequent production of more gas for heating more water. And so runs this really merry-go-round.

The globe that stands upon my desk has a long and captivating story.

Martin Behaim, the German navigator, astronomer and geographer, produced in 1492 the earliest globe of the new era. The same year Columbus sailed westward in three cockleshells and by his discovery of a western continent rendered Behaim's globe almost immediately out-of-date. When Magellan circumnavigated the earth in 1519-22, the last doubters of the earth's roundness were silenced.

The modern method of covering the globe with map *gores* or segments was evolved during the 15th Century. Leonardo Da Vinci designed a set and later Albrecht Durer worked on the mathematical problems attendant to the method. At last in 1520, a German named Schoner succeeded in covering a globe with twelve gores. Mercator, the famous map maker and originator of the most popular "flat map" projection, followed with a globe in 1541.

In the 16th Century an Englishman, Molyneux, made large globes that were the direct predecessors of modern globes. They were over 2 feet in diameter, set on a stand, encircled by a wide wood horizon ring, and at right angles to the equator, by a graduated brass meridian. A time dial capped the North Pole.

Out of this ancient, distinguished ancestry has emerged the globe as we know it today.

The glorious little tree, which in earliest spring, covers its every inch of seal-brown bark with a riot of magenta florets, long before the leaf has nerve to show itself, is the Cercis (red-bud) or Judas tree. Tradition has it that the great traitor, Judas Iscariot, hanged himself upon the branch of a kindred tree, and ever since, the Cercis shows its flushing shame at early May.

And besides the beauty of its bark and bud and blue-green cordate leaves, this species has always enjoyed a useful application. Their flowers have a very agreeable acid taste and for that reason they were used, particularly among the French Canadians, as a seasoning in salads; more often they were fried with batter, as fritters, but in their unopened condition they were frequently pickled with vinegar.

Also for many centuries the dyers of Europe and the Orient gathered the flowers for the red, blue and purple coloring substances they afforded; and it is a well-known fact that the practical French pioneers in Canada employed the bark of the one-year old twigs, which is so heavily charged with an active astringent substance, for dyeing homespun and yarn of a buff or nankeen color.

Despite the confidence which has been developed through success in using animals of all kinds, from daphnia to dog, in the testing of drugs, every once in a while something comes to shake that confidence. For instance, two scientists fed young rats three different amounts of radium chloride in daily doses. The rats got rid of most of this by excretion—at the highest dosage levels 98 to 99 per cent. of it. Yet the small amount remaining proved fatal. Although their growth appeared normal, after a short time their bones became fragile and brittle. Yet these rats were much more resistant to radium poisoning than human beings. Dr. Evans stated that the average concentration of the element in their skeletons was several hundred times greater than the concentration required to produce bone cancer in man.